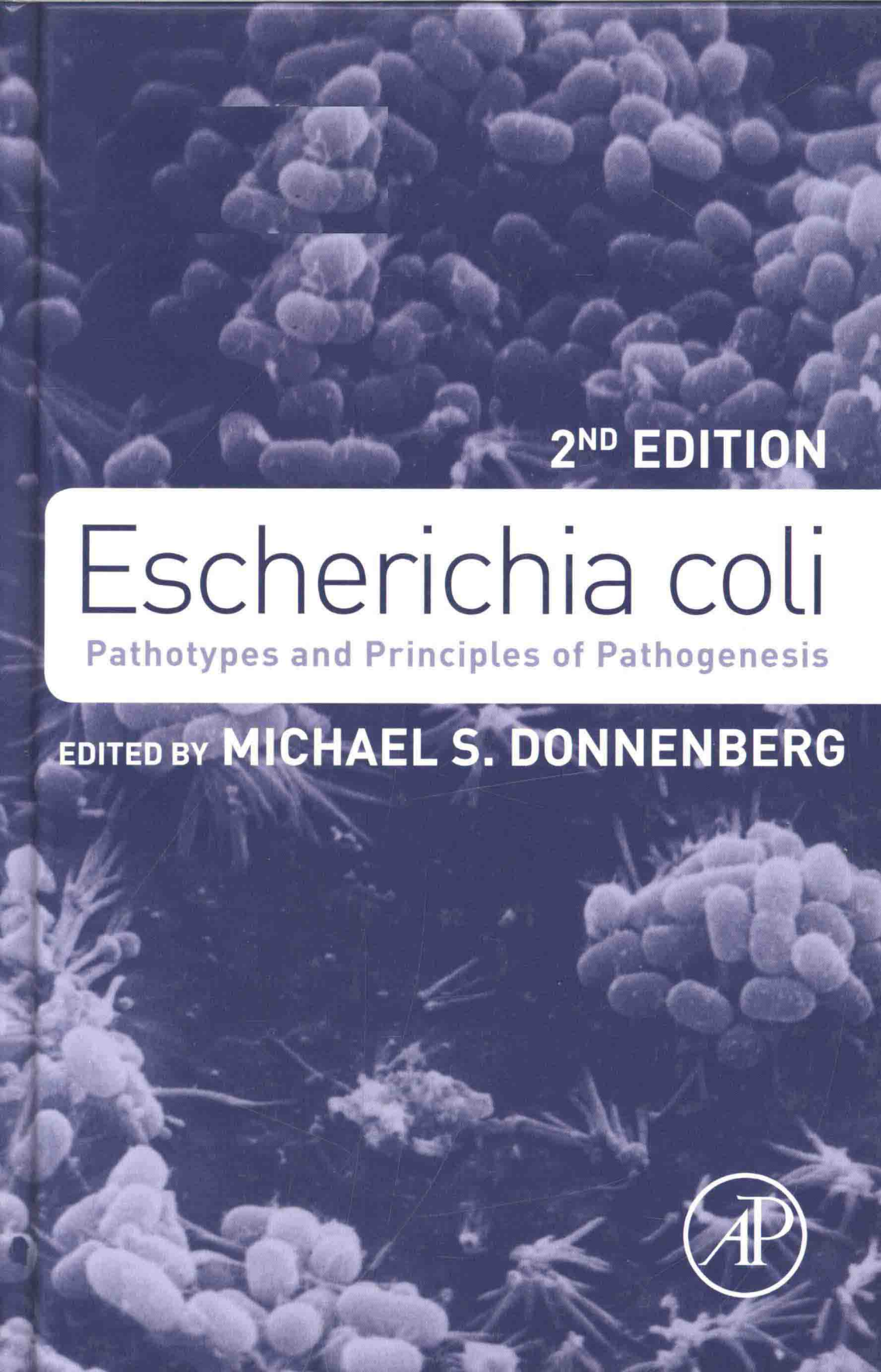
2ND EDITION
Escherichia coli
Pathotypes and Principles of Pathogenesis
EDITED BY MICHAEL S. DONNENBERG
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Escherichia coli

Pathotypes and Principles of Pathogenesis

Second Edition

Edited by

Michael S. Donnenberg
Division of Infectious Diseases
University of Maryland School of Medicine
Baltimore, Maryland, USA

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Escherichia coli

List of Contributors

Sowmya Balasubramanian Tufts University School of Medicine, Boston, MA, USA

Cedric N. Berger Imperial College London, London, UK

Julien R.C. Bergeron University of British Columbia, Vancouver, BC, Canada

Nadia Boisen University of Virginia School of Medicine, Charlottesville, VA, USA

Sujay Chattopadhyay University of Washington, Seattle, WA, USA

Abigail Clements Imperial College London, London, UK

Leon G. De Masi University of Maryland School of Medicine, Baltimore, MD, USA

Mauricio J. Farfan Universidad de Chile, Santiago, Chile

James M. Fleckenstein Washington University School of Medicine, St. Louis, MO, USA

Gad Frankel Imperial College London, London, UK

Victor A. Garcia-Angulo University of Texas Medical Branch, Galveston, TX, USA

David M. Gordon The Australian National University, Canberra, ACT, Australia

Ian R. Henderson University of Birmingham, Birmingham, UK

Scott J. Hultgren Washington University School of Medicine, St. Louis, MO, USA

Dakshina M. Jandhyala Tufts-New England Medical Center, Boston, MA, USA

Vasilios Kalas Washington University School of Medicine, St. Louis, MO, USA

Kwang Sik Kim Johns Hopkins University School of Medicine, Baltimore, MD, USA

Karen A. Krogfelt Statens Serum Institut Copenhagen, Denmark

John M. Leong Tufts University School of Medicine, Boston, MA, USA

Joshua A. Lieberman University of Maryland School of Medicine, Baltimore, MD, USA

Mariella Lomma Imperial College London, London, UK

Emily M. Mallick University of Massachusetts Medical School, Worcester, MA, USA

Anthony T. Maurelli Uniformed Services University, Bethesda, MD, USA

Harry L.T. Mobley University of Michigan Medical School, Ann Arbor, MI, USA

Carolyn R. Morris University of Maryland School of Medicine, Baltimore, MD, USA

James P. Nataro University of Virginia School of Medicine, Charlottesville, VA, USA

Shahista Nisa University of Maryland School of Medicine, Baltimore, MD, USA

David A. Rasko University of Maryland School of Medicine, Baltimore, MD, USA

Jason W. Sahl Translational Genomics Research Institute, Flagstaff, AZ, USA

Karen M. Scanlon University of Maryland School of Medicine, Baltimore, MD, USA

Evgeni V. Sokurenko University of Washington, Seattle, WA, USA

Rachel R. Spurbeck University of Michigan Medical School, Ann Arbor, MI, USA

Natalie C.J. Strynadka University of British Columbia, Vancouver, BC, Canada

Courntey D. Sturey University of Maryland School of Medicine, Baltimore, MD, USA

Alfredo G. Torres University of Texas Medical Branch, Galveston, TX, USA

Sivapriya Kailasan Vanaja Tufts University School of Medicine, Boston, MA, USA

Ender Volkan Washington University School of Medicine, St. Louis, MO, USA

Timothy J. Wells University of Birmingham, Birmingham, UK

Chris Whitfield University of Guelph, Guelph, ON, Canada

Lisa M. Willis University of Guelph, Guelph, ON, Canada

Liam J. Worrall University of British Columbia, Vancouver, BC, Canada

Introduction

In his studies of the neonatal and infant fecal flora, Theodore Escherich (1857–1911) used the nascent techniques of bacterial isolation in pure culture, Gram staining, and fermentation reactions to identify 19 bacterial species (Shulman et al., 2007). He aptly chose the designation *Bacterium coli commune* (the common colon bacterium) for the organism that now bears his name, indeed the most common facultative anaerobe in the intestinal tract of humans and many other endothermic species. As he noted, *Escherichia coli* colonizes neonates within hours of birth, an event that probably occurs during delivery as these initial strains are usually serologically identical to those found in the mother (Bettelheim et al., 1974). We remain colonized with *E. coli* bacteria throughout life, although particular strains come and go over time. Most of these strains are non-pathogenic, coexisting in harmony with their hosts. Indeed, the relationship may be symbiotic, in that the bacteria, in addition to benefiting from the host, synthesize cofactors and contribute to colonization resistance against pathogenic organisms.

This pacific image of *E. coli* belies the fact that this species can also be regarded as the prototypical pleuripotent pathogen capable of causing a wide variety of illnesses in a broad array of species. The gastrointestinal tract, the meninges, and the kidneys are among the organs targeted by *E. coli*. Diseases resulting from *E. coli* infections include diarrhea, dysentery, pyelonephritis, and the hemolytic-uremic syndrome. Outcomes include sepsis, renal failure, and death. How is it possible for this Jekyll and Hyde species to both coexist peacefully with its host and cause devastating illness?

The answer lies in the existence of different strains of *E. coli* with variable pathogenic potential. Indeed, as early as 1897, Lesage postulated this point of view (cited in Robins-Browne, 1987) and the concept ultimately achieved general acceptance when Bray established that strains that we now term enteropathogenic *E. coli* (EPEC) cause devastating outbreaks of neonatal diarrhea (Bray, 1945). Since then, a plethora of pathogenic *E. coli* varieties or pathotypes has been described. The goal of this book, now in its second edition, is to review the current state of knowledge regarding those pathotypes which cause disease in humans, placing particular emphasis on mechanisms shared among strains.

The differences in the ability of strains to cause disease and the diverse syndromes caused by the various pathotypes can be attributed to specific genes encoding virulence factors and to the capacity of *E. coli* for genetic exchange. The core *E. coli* genome, that portion shared among all strains of the species,

amounts to only about 20% of its average genome size (Rasko et al., 2008). In contrast, the total pool of genes available to be sampled by *E. coli* is much larger, at least six times that amount. Genes are constantly acquired and exchanged through plasmid transfer, bacteriophages, and perhaps by mechanisms unknown, to be tested by evolution. More subtle pathoadaptive mutations also contribute to disease. The diversity of *E. coli* and the pressures and outcomes of evolutionary forces are the focus of the first section of this book. The population structure and ecology of *E. coli* in humans, animals, and the environment is explored in the first chapter. Chapter 2 tackles the rapidly expanding universe of *E. coli* genome sequences, bringing some order to the genetic traits that define, contrast, and obscure the distinctions among pathotypes and placing these issues in the context of the radiation of *E. coli* strains from their most recent common ancestor millions of years ago. In Chapter 3 more emphasis is placed on evolutionary forces that drive the continual changes in *E. coli* genomes and the emergence of new variants capable of causing disease.

The pathogenic potential of a particular *E. coli* strain depends on the repertoire of the specific virulence genes it may possess. Particular virulence gene combinations define specific pathotypes of *E. coli*, and each pathotype has a propensity to cause a limited variety of clinical syndromes. A number of forces conspire to challenge clinicians and microbiologists to remain current in their appreciation of the diversity of *E. coli* infections. The complexity of the nomenclature is a product of the number of *E. coli* pathotypes, the similarities of their names, inconsistencies in usage in the literature, advances in our understanding of evolution and pathogenesis, and the emergence of new pathotypes. This nomenclature may be viewed as existing in a state of flux as new strains are described and the relationships among previously described pathotypes are clarified. Figure I.1 represents an attempt to illustrate these complex relationships. It is useful to view pathogenic strains as belonging to two groups: those which primarily cause gastrointestinal illness and those which primarily cause extraintestinal infections. However, there are strains with virulence potential that bridge these boundaries. Among the extraintestinal strains, it seems likely that most, if not all, strains capable of causing neonatal meningitis also can cause urinary tract infections, although the converse does not appear to be true. Among the gastrointestinal pathotypes, the situation is even more complex, especially given the overlap in attributes of EPEC and Shiga-toxin-producing *E. coli* (STEC) and the transmission of stx genes by transduction. However, a precise lexicon remains possible within the classification scheme presented. In the introduction to the first edition of this book, it was predicted that new strains would emerge with traits attributed to more than one of the pathotypes described then. Indeed, the 2011 outbreak of severe disease caused by a Shiga-toxin-producing enteroaggregative *E. coli* (Frank et al., 2011) validated this view and Figure I.1 has been updated to include such strains.

The second part of this book contains chapters detailing the molecular pathogenesis of infections due to each of the major *E. coli* pathotypes that cause human disease. These chapters provide a detailed profile of each of these

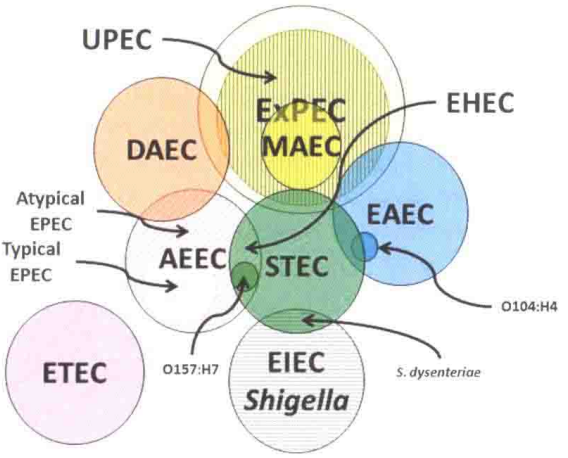

FIGURE I.1 Venn diagram illustrating the complex relationships among different pathotypes of *E. coli* that cause disease in humans. Extraintestinal pathogenic *E. coli* (ExPEC, yellow) strains include meningitis-associated *E. coli* (MAEC, bright yellow) and uropathogenic *E. coli* (UPEC, vertical stripes) and strains from patients with pneumonia, cholecystitis, peritonitis, and other infections. These strains share many virulence factors, and it is clear that single clones can cause both meningitis and urinary tract infections (Russo and Johnson, 2000). It is less clear whether or not strains exist that are capable of causing one syndrome and not the other. Among the UPEC, some strains exhibit diffuse adherence to tissue culture cells and share with diffuse adhering *E. coli* (DAEC, orange) the same adhesins. DAEC is a heterogeneous pathotype that has been epidemiologically linked to diarrhea. There are reports of DAEC strains recovered from individuals with both urinary tract infections (UTIs) and diarrhea (Germani et al., 1997). There are also reports of Shiga-toxin-producing *E. coli* (STEC, green) strains causing UTI (Tarr et al., 1996) and other extraintestinal infections. STEC are defined by production of Shiga toxins, usually encoded by bacteriophages. Among STEC, some strains are also capable of attaching intimately to epithelial cells, effacing microvilli, and eliciting the formation of adhesion pedestals composed of cytoskeletal proteins, a property that defines the attaching and effacing *E. coli* (AEEC, diagonal stripes). Strains, which are both STEC and AEEC, are known as enterohemorrhagic *E. coli* (EHEC). The most important serotype found within the EHEC pathotype is O157:H7. AEEC strains that do not produce Shiga toxins are referred to as enteropathogenic *E. coli* (EPEC). Among EPEC, many strains produce a bundle-forming pilus and attach to tissue culture cells in a localized adherence pattern. These are referred to as typical EPEC (checkered), whereas those which produce neither Shiga toxins nor bundle-forming pili are known as atypical EPEC. Some strains of atypical EPEC exhibit diffuse adherence. Enteroinvasive *E. coli* (EIEC, horizontal stripes) invade tissue culture cells with high efficiency, multiply in the cytoplasm, and spread from cell to cell. These strains include the organisms commonly classified in the genus *Shigella*, which in fact all lie phylogenetically within the species *E. coli*. Strains classified as *S. dysenteriae* serogroup 1 produce Shiga toxins and therefore could be described as members of both the EIEC and STEC pathotypes. Enteroaggregative *E. coli* (EAEC, blue) cause acute and persistent diarrhea and are defined by their pattern of adherence. In 2011 a large outbreak of severe diarrhea was caused by EAEC belonging to serotype O104:H4 that produced Shiga toxins, but other O104:H4 EAEC strains do not. Although not commonly recognized as an extraintestinal pathogen, an outbreak of community acquired UTI in Copenhagen was caused by EAEC (Olesen et al., 2012). Enterotoxigenic *E. coli* (ETEC, violet) strains cause acute diarrhea and are defined by production of heat-labile and/or heat-stable enterotoxins.

categories of organisms. It should be recognized that additional pathogenic varieties exist that cause disease exclusively in non-human species. A remarkable feature of this section is the number of distinct molecular pathways to human disease that may be employed by *E. coli*. A chapter devoted to strains that are

hybrids of other pathotypes and strains for which the pathogenic potential in humans is less well established emphasizes the dynamic nature of an evolving field. Many of these pathogenic strategies are employed by other species that cause disease in animals and humans. Thus *E. coli* can serve as a model organism for the study of bacterial pathogenesis as well as intermediary metabolism.

Despite our attempts to distinguish strains of *E. coli*, there is much overlap in the mechanisms of pathogenesis for various pathotypes. Similar virulence pathways may be pursued by more than one type of strain. For example, pili of the chaperone-usher family are ubiquitous among pathogenic and non-pathogenic *E. coli* strains. Type 3 secretion systems (T3SSs) play an important role in the pathogenesis of EPEC, enterohemorrhagic *E. coli* (EHEC), and enteroinvasive *E. coli* infections. Type IV pili and the closely related type 2 secretion systems are expressed by EPEC and enterotoxigenic *E. coli* (ETEC). Hemolysins of the RTX family are produced by many strains of *E. coli* that cause extraintestinal infections, by EHEC, and occasionally by other strains associated with intestinal infections, while proteins exported by the autotransporter or type 5 pathway are ubiquitous among *E. coli*. Many strains of pathogenic *E. coli*, especially those that cause extraintestinal infections, elaborate polysaccharide capsules, and all strains make lipopolysaccharide. To allow these critical virulence factors to be explored in more detail than is possible in the second section, the final part of this book contains chapters devoted to virulence systems that are common to more than one pathotype. The explosion of information on the structure and function of T3SSs and the function of effector proteins employed by more than one pathotype are explored in two separate chapters. By design, each chapter of this book can stand alone while references among the chapters allow the reader to explore further detail on virulence mechanisms and how different pathotypes exploit similar systems.

The interrelationships among various pathogenic and non-pathogenic *E. coli* strains, the complexities of the disease pathways navigated by each pathotype, and the overlap in virulence mechanisms employed by different types reveal an intricate web of information about the organism. Yet there remains much to learn. Despite our advances in the cellular and molecular details of the interactions between these organisms and host cells, we remain ignorant of the mechanisms by which most strains of *E. coli* actually cause disease. Interactions with host factors likely dictate outcome for many infections. For some pathotypes, the virulence mechanisms that define the group remain largely mysterious. For other pathotypes that we thought we understood, further research has revealed new surprises, the significance of which has yet to be fully realized. The ever-increasing availability of genomic sequences will continue to reveal unanticipated genes that may help to unravel disease mechanisms, clarify relationships among pathotypes, and provide insight into the evolution of the species. It remains likely that additional pathotypes of *E. coli* lurk unrecognized, awaiting characterization until new assays are applied to strains isolated from patients and controls. *E. coli* has been subject to intensive scrutiny for more than a century and will continue to be regarded with interest for a long time to come.

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